Environment and Natural Resources Trust Fund
2016 Request for Proposals (RFP)

Project Title: **ENRTF ID: 040-B**

Quantifying Bacteria for Better Wastewater Treatment Process Control

Category: B. Water Resources

Total Project Budget: $ 398,592

Proposed Project Time Period for the Funding Requested: 3 years, July 2016 to June 2019

Summary:

This project will characterize and quantify the nutrient-removing microorganisms used for municipal wastewater treatment to help provide better process control, as needed to meet future regulations.

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Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Wastewater treatment protects our rivers, lakes and oceans from the harmful effects of sewage. New regulations on Minnesotas wastewater treatment facilities are anticipated in the near future for total nitrogen emissions, which will pose a significant challenge for the States treatment plants to meet. The proposed research will help develop and apply new methods to quantify the microorganisms that treat the wastewater, thus leading to significant improvements in process control.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: *Quantifying bacteria for better wastewater treatment process control*

I. PROJECT STATEMENT

In the near future (5-10 years), new regulations are expected on Minnesota's municipal wastewater discharges for total nitrogen (ammonia, nitrite, and nitrate), which is needed to prevent the eutrophication of the Gulf of Mexico. The goal of this proposed project is to understand the composition of the microbial communities used for municipal wastewater treatment and to provide baseline information of the quantities of nutrient-removing microorganisms used for wastewater treatment in the State of Minnesota. The benefit of this research will be a useful set of tools that can be used to better control wastewater treatment operations.

All wastewater treatment facilities in Minnesota are currently regulated with respect to the release of biodegradable organic compounds (known as "BOD" – biochemical oxygen demand). These regulations ensure that the harmful impacts of wastewater are avoided, particularly the consumption of oxygen in the receiving water body (oxygen is needed for fish and other aquatic fauna and flora to thrive). Presently, many wastewater treatment facilities are also seasonally regulated for ammonia (due to fish toxicity) and continuously regulated for phosphorus, which contributes to the eutrophication (the excessive growth of algae) in lakes.

The most cost-effective way to treat municipal wastewater is to utilize microorganisms to metabolize pollutants of concern. Wastewater treatment facilities use microorganisms to remove the nutrients (BOD, phosphorus, nitrogen) of concern from the wastewater. From an engineering and operational perspective, it is relatively straight-forward to achieve either BOD and phosphorus removal or BOD and total nitrogen removal, but it is **much more difficult problem** to achieve BOD, total nitrogen, and phosphorus removal because all three processes occur only over a very narrow range of operating conditions. Future wastewater treatment operations, therefore, will likely require better process control; the research proposed herein will delineate the tools needed to provide this better process control.

Surprisingly, wastewater treatment bioreactors are currently operated with very little knowledge of the microorganisms that provide the treatment. The scientific reason for the lack of monitoring has been the inability of microbiologists to culture organisms from environmental samples. Over the past 5-10 years, however, microbiologists have developed next-generation DNA sequencing technology to generate 50,000+ sequences per sample to address the question of "*who is there*?" In addition, quantitative polymerase chain reaction (qPCR) can be used to determine the precise quantities of specific organisms, such as those responsible for phosphorus and nitrogen removal, allowing us to address "*how many of them are there*?"

Of particular importance, qPCR techniques are now relatively affordable (less than $30,000 for all of the needed instrumentation – on par with other lab techniques) and practical, requiring no special skills beyond those possessed by typical laboratory technicians. The final activity will be to disseminate the research results to Minnesota's wastewater treatment plant managers and operators (and providing training, if requested), with the goal of using these new technologies for better wastewater treatment process control.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: *Characterize Minnesota's wastewater treatment microbiome.* **Budget: $194,017**

Samples will be collected from the bioreactors treating wastewater and wastewater sludges at selected wastewater treatment facilities on a weekly basis. We will specifically collect samples from the wastewater treatment facilities both in St. Cloud and in Brainerd, because these two facilities were recently upgraded to perform simultaneous removal of BOD, nitrogen, and phosphorus. We will also collect samples from wastewater treatment facilities in Little Falls, Duluth, St. Peter, and Mankato; these facilities do not currently perform simultaneous BOD, nitrogen, and phosphorus removal. Next-generation DNA sequencing will be performed using an Illumina MiSeq analyzer at the University of Minnesota Genomics Center. We will then use software that is available at the Minnesota Supercomputing Institute to statistically analyze the data and correlate our data to process performance (which is routinely collected from each facility).

Outcome	Completion Date
1. Sample collection and Genomic DNA extractions (4,200 samples)	December 31, 2017
2. Next-Generation DNA sequencing (20 Illumina MiSeq runs)	June 30, 2018
3. Data Analysis at the Minnesota Supercomputing Institute	April 30, 2019

Activity 2: *Quantify nutrient-removing microbial populations in wastewater bioreactors.* **Budget: $194,017**

A very similar process will be used for Activity 2 as for Activity 1. In this activity, however, we will quantify the presence of specific microbial populations rather than track the identity all microorganisms. We will specifically perform qPCR targeting ammonia-oxidizing bacteria, ammonia-oxidizing archaea, denitrifying bacteria (3 different types), methanogenic archaea, phosphate-accumulating organisms, and total bacteria. We will perform these assays using the 384-well real time PCR system at the University of Minnesota Genomics Center (approximately 35,000 quantifications).

Outcome	Completion Date
1. Sample collection and Genomic DNA extractions	December 31, 2017
2. qPCR targeting specific nutrient-removing microorganisms	June 30, 2018
3. Data Analysis at the Minnesota Supercomputing Institute	April 30, 2019

Activity 3: *Disseminate our results to Minnesota's wastewater treatment facilities.* **Budget: $10,558**

The first two activities will demonstrate the value of tracking bacterial populations in Minnesota's wastewater treatment facilities. The final activity will be to disseminate these results at local conferences and then to work with Minnesota's wastewater treatment facilities to perform these assays in-house.

Outcome	Completion Date
1. Presentations at local wastewater treatment conferences	December 31, 2018
2. Train laboratory technicians at wastewater treatment facilities to use qPCR	June 30, 2019

III. PROJECT STRATEGY

A. Project Team/Partners:

The project will be led by Professors Timothy LaPara and Sebastian Behrens (University of Minnesota, Department of Civil, Environmental, and Geo- Engineering). The team will also consist of a post-doctoral researcher and one graduate student researcher. Drs. LaPara and Behrens are experts in biological wastewater treatment and analyzing complex microbial communities. The graduate student, Julie Johnston, was recently awarded a graduate fellowship by the National Science Foundation; as such, two of the three years that she will work on the proposed project will be provided by federal funds.

B. Project Impact and Long-Term Strategy:

This project will provide critically important scientific knowledge on the microorganism that the State of Minnesota depends on to treat its numerous municipal wastewater streams. Although the State currently does an excellent job meeting its current discharge regulations, future regulations on BOD, nitrogen, and phosphorus will pose a much more difficult challenge that will require much more detailed knowledge of the microorganisms used during wastewater treatment (specifically the organisms involved in nitrogen and phosphorus removal). This proposed project will help meet that critical need.

C. Timeline Requirements:

The proposed project will be completed in a three-year period. Samples will be collected for the first 18 months of the project followed by microbiological analyses that are time consuming.

2016 Detailed Project Budget

Project Title: *Quantifying bacteria for better wastewater treatment process control*

IV. TOTAL ENRTF REQUEST BUDGET 3 years

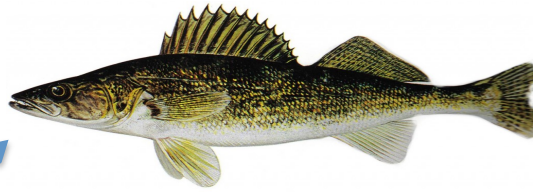
BUDGET ITEM	AMOUNT
Personnel:	
Timothy M. LaPara, Project Manager (75% salary, 25% benefits); 8% FTE; Project supervision, supervision of post-doctoral and graduate student, project reporting, dissemination and outreach.	$ 48,808
Sebastian Behrens, co-Project Manager (75% salary, 25% benefits); 8% FTE; Project supervision, supervision of post-doctoral and graduate student, project reporting, dissemination and outreach.	$ 44,292
Post-doctoral researcher, to be hired (82.4% salary, 17.6% benefits); 100% FTE; Collect samples, perform next-generation DNA sequencing, and quantify nutrient-removing bacteria	$ 171,041
Julie Johnston, graduate researchers (100% salary, 41% benefits); 50% FTE; Collect samples, perform next-generation DNA sequencing, and quantify nutrient-removing bacteria	$ 47,451
Professional/Technical/Service Contracts:	$ -
University of Minnesota Genomics Center: next generation DNA sequencing (4200 samples; 210 samples per run = 20 runs x $1500 per run) and high-throughput qPCR (15 qPCR per target x 5 targets x 375 samples per run x $100 per run)	$ 37,500
Equipment/Tools/Supplies:	
DNA extraction kits ($3 per sample; 4200 samples)	$ 12,600
Reagents for qPCR ($5 per sample; 4200 samples)	$ 21,000
DNA purification for next generation sequencing ($2 per sample; 4200 samples)	$ 8,400
Miscellaneous laboratory supplies (disposal regeants, micropipette tips, etc)	$ 3,000
Travel:	
In-state travel to collect samples and visit treatment facilities for outreach/dissemination	$ 4,500
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 398,592

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period: *Graduate student support for two of the three years of the project will be provided by a National Science Foundation fellowship to Julie Johnston*	$ 121,493	*Pending*
Other State $ To Be Applied To Project During Project Period:	N/A	

Quantifying bacteria for better wastewater treatment process control

Wastewater Treatment



Biodegradable organic carbon removal protects fish and other aquatic organisms

Nitrogen removal prevents eutrophication of coastal water

Phosphorus removal prevents eutrophication of lakes

- New regulations are expected to require the removal of biodegradable organic carbon, nitrogen, and phosphorus, which will pose a significant challenge
- This project will develop and apply assays for characterizing and quantifying the bacteria that remove carbon, nitrogen and phosphorus, leading to essential improvements in wastewater treatment process control

Project Manager Qualifications and Organization Description

Timothy M. LaPara

Professor, Department of Civil, Environmental, and Geo- Engineering, University of Minnesota

B.S.C.E., Civil Engineering, 1995, University of Notre Dame, Notre Dame, IN
Ph.D., Civil Engineering, 1999, Purdue University, West Lafayette, IN

Dr. Timothy LaPara will be responsible for overall management of the proposed project. Dr. LaPara's research is focused on the role of municipal and industrial wastewater treatment plants in preserving environmental quality and in protecting public health. His research has a strong interdisciplinary nature, stemming from his unique background in both environmental engineering and microbiology.

Sebastian F. Behrens

Associate Professor, Department of Civil, Environmental, and Geo- Engineering, University of Minnesota

B.S., Biology 1997, University of Bremen, Germany
Diploma, 2000, Microbiology, University of Bremen, Germany
Ph.D., Microbial Ecology, 2003, Max Planck Institute for Marine Microbiology, Germany

Dr. Behrens' research focuses on linking environmental processes to the spatial-temporal distribution and metabolic activity of key functional groups of microorganisms. He follows an interdisciplinary approach that combines the disciplines biogeochemistry, microbiology, and molecular biology to understand the basic microbial ecology principles driving the biogeochemical cycling of metals and metalloids, the biodegradation of organic contaminants, and the emission of greenhouse gases from the molecular to the ecosystem scale. The gained knowledge on microbial transformation processes in natural and engineered ecosystems is then implemented in order to optimize microbial remediation approaches, resource recovery, and the biological treatment of water (drinking water, surface water, groundwater, or waste water), thereby spanning the gap between basic and applied research aspects of bioremediation.

Organization Description

The University of Minnesota is one of the largest, most comprehensive, and most prestigious public universities in the United States (http://www1.umn.edu/twincities/about/index.html). The laboratories and offices of the PIs contain the necessary fixed and moveable equipment and facilities needed for the proposed studies.